Research Article
Flavour and Fragrance Journal

Received: 16 October 2009; Revised: 9 December 2009; Accepted: 21 December 2009; Published online in Wiley InterScience: 18 January 2010

(www.interscience.wiley.com) DOI 10.1002/ffj.1972

Antioxidant activity of six Portuguese thyme species essential oils†

S. Anahi Dandlen,[a] A. Sofia Lima,[b] Marta D. Mendes,[b] M. Graça Miguel,[a]* M. Leonor Faleiro,[c] M. João Sousa,[d] Luis G. Pedro,[b] José G. Barroso[b] and A. Cristina Figueiredo[b]

ABSTRACT: The essential oils of *Thymus caespititius*, *T. camphoratus*, *T. capitellatus*, *T. carnosus*, *T. pulegioides*, *T. zygis* subsp. *zygis* and *T. zygis* subsp. *sylvestris* collected in different regions of Portugal, including the Azores islands, were evaluated for preventing lipid peroxidation and scavenging free radicals, and hydroxyl and superoxide anions. *T. zygis* subsp. *zygis* oil possessed the best capacity for preventing lipid peroxidation ($IC_{50} = 0.030 \pm 0.003$ mg/ml), immediately followed by the oils isolated from *T. zygis* subsp. *sylvestris* collected in different regions of Portugal. IC_{50} values ranged from 0.066 ± 0.003 to 0.087 ± 0.001 mg/ml in oils isolated from samples from Alcanena and Duas Igrejas, respectively. The oils isolated from *T. zygis* subsp. *sylvestris*, independent of the harvesting region, were shown to be the most effective for scavenging free radicals (0.358–0.453 mg/ml). The best capacity for scavenging hydroxyl radicals was found in the oils of *T. camphoratus* collected in Espartal and of *T. caespititius* of Lordelo, with $IC_{50} < 0.5$ mg/ml. The low oil amount did not allow IC_{50} values for the superoxide anion scavenging determination, therefore considering a unique oil concentration (5 mg/ml), the highest inhibition percentages (>50%) were registered with the essential oils of *T. zygis* subsp. *sylvestris* from Duas Igrejas and Covão do Coelho, and of *T. capitellatus* from Sines. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: *Thymus*; Lamiaceae; Labiatae; antioxidant activity; essential oils

Introduction

One of the major concerns in food technology is lipid oxidation, due to the formation of oxidation products such as fatty acid hydroperoxides and secondary degradation products (alkanes, aldehydes, alkenes). The later components are responsible for off-flavours and they arise from hydroperoxy radicals formed during autoxidation. The formation of these off-flavours, with characteristic rancid odours, is responsible for the decrease of both the nutritional quality and safety of foods.[1,2] Oxidation processes are also deleterious in human health, since they induce tissue damage responsible for several pathologies, including cancer, neurodegenerative and ischaemic heart diseases, malaria, arteriosclerosis and other pathological conditions.[3]

The utilization of antioxidants can prevent food oxidation or cell damage. To prevent this degradation process of lipids, the food industry adds antioxidants of low cost and high stability, mostly synthetic ones, such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), gallats, and *tert*-butylhydroquinone (TBHQ), in well-defined concentrations. However, such compounds present some toxicity.[4]

In a recent review, Yanishlieva *et al.*[2] compiled the results of several studies that demonstrated the capacity of herbs and spices (rosemary, sage, oregano, thyme, ginger, summer savoury, black pepper, red pepper, clove, marjoram, basil, peppermint, spearmint, common balm, fennel, parsley, cinnamon, cumin, nutmeg, garlic and coriander, among others) to act as antioxidants, mainly those belonging to the family Lamiaceae. The authors also showed that the part of the plant used, the collection zone, the substrate and antioxidant method used were responsible for the diversity of results, often hampering direct comparison.

The antioxidant properties of samples (plant extracts, essential oils and pure compounds) can be evaluated using various *in vitro* assays, which can be divided in two main groups: (a) those that evaluate lipid peroxidation; and (b) those that measure free radical scavenging ability.[5]

* Correspondence to: M. Graça Miguel, Universidade do Algarve, Faculdade de Ciências e Tecnologia, CDCTPV, Campus de Gambelas, 8005-139 Faro, Portugal. E-mail: mgmiguel@ualg.pt

[a] Universidade do Algarve, Faculdade de Ciências e Tecnologia, CDCTPV, Campus de Gambelas, 8005-139 Faro, Portugal

[b] Universidade de Lisboa, Faculdade Ciências Lisboa, DBV, Instituto de Biotecnologia e Bioengenharia, Centro Biotecnologia Vegetal, C2, Campo Grande, 1749-016 Lisboa, Portugal

[c] Universidade do Algarve, Faculdade de Ciências e Tecnologia, Instituto de Biotecnologia e Bioengenharia, Centro de Biomedicina Molecular e Estrutural, Campus de Gambelas 8005-139 Faro, Portugal

[d] Instituto Politécnico de Bragança, Escola Superior Agrária, Dep. Biologia e Biotecnologia, Campus de Sta Apolónia, Apartado 1172, 5301-855 Bragança, Portugal

† This paper was published in *Flavour and Fragrance Journal* as part of the special issue based on the lectures given at the 40th International Symposium on Essential Oils, held at Savigliano, Italy, 6–9 September 2009, organized by Carlo Bicchi and Patrizia Rubiolo

In assessing lipid peroxidation, a lipidic substrate is needed and the antioxidant activity can be detected by measuring substrate and oxidant consumption and the intermediates or the final products formation. Most tests based on a lipid substrate need accelerated oxidation conditions, such as increased partial oxygen pressure and temperature, addition of transition metal catalysts, exposure to light, variable shaking and free radical sources.[6]

In measuring free radical scavenging ability, two group methods are considered, according to the chemical reactions involved: hydrogen atom transfer reaction-based methods, and single electron transfer reaction-based methods.[7,8] In addition, there are tests that evaluate the effectiveness against several reactive oxygen species and nitrogen reactive species ($O_2^{\bullet -}$, $HO^{\bullet}$, $ONOO^-$, H_2O_2), which are also needed and generally performed. As all methods are based on different chemical and physical principles of oxidation monitoring, the antioxidant activities may change, depending on the method followed.[9] Therefore the difficulty for comparing antioxidant activities can be attributed to a lack of reference method(s) expressed in adequate and universal units.

In a recent review article, Figueiredo *et al.*[10] compiled the available data on the antioxidant activity of Portuguese thyme essential oils, mainly for preventing lipid peroxidation, with reference to how such activities depend on the chemical composition of samples; evaluation method and type of lipid substrate used.

In the present study, the capacity of the essential oils of *T. caespititius*, *T. camphoratus*, *T. capitellatus*, *T. carnosus*, *T. pulegioides*, *T. zygis* subsp. *zygis* and *T. zygis* subsp. *sylvestris* collected in different regions of Portugal, including the Azores islands, for preventing lipid peroxidation and scavenging free radicals, hydroxyl and superoxide anion was evaluated.

Experimental

Plant Material

The aerial parts of *T. caespititius*, *T. camphoratus*, *T. capitellatus*, *T. carnosus*, *T. pulegioides*, *T. zygis* subsp. *zygis* and *T. zygis* subsp. *sylvestris* were collected during the flowering phase in different regions of Portugal and dried in a dark place at room temperature. Voucher specimens have been deposited in the Herbarium of the Instituto Botânico da Faculdade de Ciências de Lisboa.

Isolation of Essential Oils

The essential oils were isolated from the dried plant material by hydrodistillation for 3 h, using a Clevenger-type apparatus, according to the *European Pharmacopoeia* method.[11] The essential oils were stored at −20°C in the dark prior to analysis.

Chemical Analysis of the Essential Oils

Gas chromatography (GC). Gas chromatographic analyses were performed using a Autosystem XL (Perkin Elmer, Shelton, CT, USA) gas chromatograph equipped with two flame ionization detectors (FIDs), a data-handling system and a vapourizing injector port, into which two columns of different polarities were installed: a DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm; J&W Scientific, Rancho Cordova, CA, USA) and a DB-17HT fused-silica column (30 m × 0.25 mm i.d., film thickness 0.15 μm; J&W Scientific). Oven temperature was programmed from 45°C to 175°C at 3°C/min, then at 15°C/min to 300°C, then held isothermal for 10 min; injector and detector temperatures, 280°C and 300°C, respectively; carrier gas, hydrogen, adjusted to a linear velocity of 30 cm/s. The samples were injected using the split sampling technique, ratio 1:50. The volume of injection was 0.1 μl of a pentane–oil solution. The relative percentages of the main constituents are only indicative.

Gas chromatography–mass spectrometry (GC–MS). The GC–MS unit consisted of a Autosystem XL (Perkin-Elmer) gas chromatograph, equipped with a DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm; J&W Scientific) and interfaced with a Turbomass mass spectrometer (software v. 4.1, Perkin-Elmer). Injector and oven temperatures were as above; transfer line temperature, 280°C; ion trap temperature, 220°C; carrier gas, helium, adjusted to a linear velocity of 30 cm/s; split ratio, 1:40; ionization energy, 70 eV; ionization current, 60 μA; scan range, 40–300 u; scan time, 1 s. The identity of the components was assigned by comparison of their retention indices, relative to C_9–C_{16} *n*-alkane indices and GC–MS spectra from a home-made library, constructed based on analyses of reference oils, laboratory-synthesized components and commercially available standards.

Antioxidant Activity Evaluation

Thiobarbituric acid-reactive substances (TBARS). The experiment was based on a modified thiobarbituric acid (TBA) reactive substances assay (TBARS) to measure the antioxidant ability of the samples. Egg yolk homogenate was used as the lipid-rich medium, obtained as described by Dorman *et al.*,[12] i.e. an aliquot of yolk material was made up to a concentration of 10% w/v in KCl (1.15% w/v). The yolk was then homogenized for 30 s followed by ultrasonication for further 5 min. Homogenate (500 μl) and 100 μl sample or positive control (BHT), dissolved in methanol, were added to a test tube and made up to 1 ml with distilled water, followed by the addition of 1.5 ml 20% acetic acid, pH 3.5, and 1.5 ml 0.8% w/v TBA in 1.1% w/v sodium dodecyl sulphate (SDS). This mixture was stirred in a vortex and heated at 95°C for 60 min. After cooling to room temperature, 5 ml *n*-butanol was added to each tube, stirred and centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (Shimadzu 160-UV). The percentage of inhibition was calculated using the following equation:

$$\text{Inhibition} = [(A_0 - A_1)/A_0] \times 100(\%)$$

where A_0 is the absorbance of the control (without sample) and A_1 is the absorbance in the presence of the sample. Tests were carried out in triplicate. The sample concentration providing 50% inhibition (IC_{50}) was obtained by plotting the inhibition percentage against essential oil concentrations.

Free Radical Scavenging Activity (DPPH)

A methanolic stock solution (50 μl) of each sample (essential oils) and positive control (BHT) at different concentrations was placed in a cuvette, and 2 ml 60 μM methanolic solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) was added.[13] Absorbance measurements were made at 517 nm, using a Shimadzu 160-UV spectrophotometer (Tokyo, Japan) after 60 min of reaction at room temperature. Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the negative control. The percentage inhibition of the DPPH radical by the samples was calculated according to the following formula:

$$\text{Scavenging effect} = [(A_0 - A_1)/A_0] \times 100(\%)$$

where A_0 is the absorbance of the blank sample and A_1 is the absorbance in the presence of the sample. Tests were carried out in triplicate. The sample concentration providing 50% inhibition (IC_{50}) was obtained by plotting the inhibition percentage against essential oil concentrations.

Hydroxyl Radical Scavenging Activity

The assay of •OH-scavenging activity was developed according to Chung *et al.*,[14] with small modifications. Briefly, the reaction mixture was prepared with 10 mM $FeSO_4.7H_2O$, 10 mM EDTA, 10 mM 2-deoxyribose, 0.1 M phosphate buffer and sample or positive control (mannitol) in a test tube to give a total volume of 1.8 ml. Finally, 200 μl H_2O_2 was added to the mixture, which was incubated at 37°C for 4 h. After that, 1 ml trichloroacetic acid (2.8%) and 1 ml thiobarbituric acid (1%) were added to the test tube, which was boiled for 10 min. After cooling, its absorbance was measured at 520 nm in a Shimadzu 160-UV spectrophotometer. The •OH-scavenging activity (%) was calculated using the following equation:

$$\text{Inhibition} = [(A_0 - A_1)/A_0] \times 100(\%)$$

where A_0 is the absorbance of the control (without sample) and A_1 is the absorbance in the presence of the sample. Tests were carried out in triplicate. The sample concentration providing 50% inhibition (IC_{50}) was obtained by plotting the inhibition percentage against essential oil concentrations.

Superoxide Anion Scavenging Activity

Measurements of the superoxide anion scavenging activity of samples were based on the method described by Payá *et al*.[15] Superoxide anions were generated in an enzymatic hypoxanthine/xanthine oxidase system assayed by the reduction of nitroblue tetrazolium (NBT). The superoxide anion was generated in 666 μl phosphate buffer (KH_2PO_4, 50 mM, pH 7.4), containing 100 μl hypoxanthine 1 mM, 100 μl EDTA 1 mM, 100 μl NBT 1 mM, and different concentrations of samples or positive control (gallic acid). The reaction was started by the addition of 31.5 μl xanthine oxidase (0.73 U/ml) to the mixture. The absorbance was recorded at 560 nm against blank samples in a Shimadzu 160-UV spectrophotometer. The percentage of inhibition was calculated using the following equation:

$$\text{Inhibition} = [(A_0 - A_1)/A_0] \times 100(\%)$$

where A_0 is the absorbance of the control (without sample) and A_1 is the absorbance in the presence of the sample.

Statistical Analysis

Statistical comparisons were made with one-way ANOVA, followed by Tukey multiple comparison test. The level of significance was set at $p < 0.01$. Statistical calculations were performed using SPSS 15.0 software.

Results and Discussion

Antioxidant Activity

Thiobarbituric acid reactive substances (TBARS). The best antioxidant activity, as assessed by the TBARS method, was obtained with *T. zygis* subsp. *zygis* oil from Rebordãos (0.030 mg/ml). All *T. zygis* subsp. *sylvestris* oils, with IC_{50} = 0.066–0.087 mg/ml, also showed very good antioxidant capacity, significantly higher than the remaining samples (Table 1). Both oils showed better activity than BHT (IC_{50} = 0.173 mg/ml).

Although several of the essential oils that showed the highest antioxidant activity were carvacrol-, thymol- or *p*-cymene-rich oils (Table 1), no straight correlation can be drawn between one sole main component and antioxidant activity. These results support the view that antioxidant activity is a consequence of the synergistic activity between essential oil components, and that being a phenolic-terpene rich oil is not a synonym for having antioxidant activity.

1,8-Cineole-, camphene- and borneol-rich oils, such as those of *T. camphoratus* and *T. carnosus* oils (Table 1), showed significantly lower capacity to prevent lipid oxidation than *T. zygis* and *T. caespititius* oils. Nevertheless, similarly to what was found for phenolic-terpene rich oils, 1,8-cineole-, camphene- and borneol-rich oils showed significant variability of antioxidant activities, that can only be attributable to synergistic or antagonistic effects among essential oil components.

Free Radical Scavenging Activity (DPPH)

The free radical scavenging activity of some oils was not determined, due to an insufficient amount of oil. *T. zygis* subsp. *sylvestris* phenolic-terpene-rich oils were the most effective for scavenging DPPH radicals, with $IC_{50} < 0.5$ mg/ml (Table 1), which was nevertheless always inferior to the positive control (BHT), with $IC_{50} = 0.013$ mg/ml. The IC_{50} values found in our research are comparable to those reported by other authors working with other *Thymus* species[16–19] and significantly higher than that of *Nigella sativa* oil, in which *p*-cymene predominated.[20]

Hydroxyl Radical Scavenging Activity

The essential oils of *T. camphoratus* from Espartal (0.417 mg/ml), *T. caespititius* from Lordelo (0.465 mg/ml) and *T. capitellatus* from Estr. Sines-Grândola (0.508 mg/ml), were significantly more effective to scavenger hydroxyl radicals than the remaining oil samples (Table 1) and the positive control (mannitol) with IC_{50} = 1.940 mg/ml. Borneol, camphor, α-terpineol, 1,8-cineole, camphene, α-pinene and *p*-cymene constituted the major compounds of these essential oils in different relative amounts. However, there were also essential oils of some *Thymus* species (*T. caespititus* from Terras do Bouro, Vilarinho das Furnas, Caramulo; and *T. capitellatus* from Carvalhal and Alcácer do Sal) in which borneol, α-terpineol, 1,8-cineole and *p*-cymene predominated, and they showed the lowest hydroxyl radicals scavenger activity, with IC_{50} > 1.0 mg/ml.

From these results, it seems that for scavenging hydroxyl radicals, the presence of phenolic compounds (thymol or carvacrol) in the oil is not determinant, since the oils in which these components predominated did not show great activities. On the other hand, those oils in which borneol, camphor, α-terpineol, 1,8-cineole, camphene, α-pinene and *p*-cymene predominated showed either the highest or the lowest activities, depending on the species and the harvesting place.

Superoxide Anion Scavenging Activity

Only the essential oils from *T. zygis* subsp. *silvestris* from Covão do Coelho (50%) and Duas Igrejas (59%), and from *T. capitellatus* from Estr. Sines-Grândola (52%) showed a superoxide anion scavenging activity >50% (Table 1). In both samples of *T. zygis*, there was always at least one phenolic compound predominant in the oil (carvacrol or thymol), whereas in *T. capitellatus*, borneol and 1,8-cineole prevailed. Other carvacrol- or thymol-rich oils showed lower activity than those of *T. zygis*, with scavenging percentages in the range 23–44% (*T. caespititius*, from Planalto Central, and *T. zygis* subsp. *zygis* from Condeixa, respectively). Such activities were significantly lower when compared to that of the positive

Table 1. *Thymus* species studied, their harvesting collection site, four main essential oils components, oil yields and corresponding antioxidant activities assessed by four different methodologies

Thymus species	Harvesting place	Four main essential oil components (%)	Oil yield (% v/w)	TBARS	DPPH IC_{50} (mg/ml)	Hydroxyl radical	Superoxide anion inhibition (%)
Thymus caespititius Brot.	Caramulo (MP)	α-Terpineol (40.3), *p*-cymene (13.8), γ-terpinene (5.4), τ-cadinol (5.2)	0.8	0.200 ± 0.011 bc	nd	1.188 ± 0.002 jk	19.3 ± 0.3 c
	Lordelo (MP)	α-Terpineol (23.5), *p*-cymene (15.9), γ-terpinene (11.7), τ-cadinol (6.9)	0.7	0.240 ± 0.018 cd	nd	0. 465 ± 0.004 ab	21.8 ± 0.8 e
	Óbidos (MP)	α-Terpineol (51.5), *p*-cymene (14.5), γ-terpinene (6.5), τ-cadinol (6.2)	2.3	0.326 ± 0.017 cde	0.607 ± 0.093 ab	0.691 ± 0.010 cd	27.6 ± 0.3 hi
	Outeiro (MP)	α-Terpineol (40.5), *p*-cymene (13.7), γ-terpinene (8.7), β-caryophyllene (2.7)	0.8	0.520 ± 0.029 f	nd	0.935 ± 0.002 fgh	21.6 ± 0.3 de
	Pico (A)	Carvacrol (50.7), carvacryl acetate (18.7), *p*-cymene (5.7), γ-terpinene (3.8)	0.4	0.205 ± 0.021 bc	nd	0.887 ± 0.002 ef	32.8 ± 0.7 k
	Pico Verde (A)	Carvacrol (32.2), thymol (23.0), carvacryl acetate (7.0), *p*-cymene (5.9)	1.0	0.357 ± 0.017 def	nd	1.442 ± 0.014 o	32.2 ± 0.8 k
	Planalto Central (A)	Carvacrol (61.9), carvacryl acetate (11.5), α-terpineol (3.0), *p*-cymene (2.6)	0.6	0.345 ± 0.023 def	2.341 ± 0.025 cd	0.715 ± 0.008 cd	22.5 ± 0.6 ef
	Ponta dos Rosais (A)	Thymol (24.9), α-terpineol (19.1), *p*-cymene (11.5), γ-terpinene (9.6)	0.8	0.352 ± 0.029 def	1.847 ± 0.157 bc	0.996 ± 0.016 hij	25.9 ± 0.2 gh
	Serra do Cume (A)	Thymol (34.8), carvacrol (12.7), *p*-cymene (8.2), thymyl acetate (7.9)	0.6	0.460 ± 0.011 ef	0.740 ± 0.100 ab	1.032 ± 0.011 j	25.3 ± 0.8 g
	Terras de Bouro (MP)	α-Terpineol (24.1), γ-terpinene (13.8), *p*-cymene (12.3), γ-eudesmol (6.2)	0.6	0.323 ± 0.028 cde	nd	1.280 ± 0.002 mn	23.7 ± 0.4 f
	Vilarinho das Furnas (MP)	α-Terpineol (42.4), *p*-cymene (13.5), γ-terpinene (6.2), β-caryophyllene (3.7)	0.6	0.699 ± 0.016 g	nd	1.247 ± 0.216 lm	19.5 ± 0.3 c
Thymus camphoratus Hoffmanns. & Link	Atalaia (MP)	1,8-Cineole (46.7), linalool (12.2), linalyl acetate (8.8), α-pinene (4.3)	0.9	2.190 ± 0.083 p	2.263 ± 0.075 cd	1.006 ± 0.060 hij	15.0 ± 0.3 b
	Boca do Rio (MP)	1,8-Cineole (26.5), borneol (15.0), α-pinene (12.3), camphene (11.6)	1.1	2.004 ± 0.348 o	3.217 ± 0.164 de	0.668 ± 0.011 c	22.3 ± 0.5 ef
	Cabo de S. Vicente (MP)	1,8-Cineole (37.0), α-pinene (10.1), terpinen-4-ol (9.8), borneol (4.3)	0.7	0.688 ± 0.016 g	1.560 ± 0.031 abc	0.690 ± 0.001 cd	27.2 ± 1.1 hi
	Espartal (MP)	Borneol (23.2), camphor (19.1), camphene (17.2), linalool (9.5)	1.9	1.246 ± 0.081 m	1.962 ± 0.014 bcd	0.417 ± 0.006 a	16.3 ± 0.4 b

Flavour Fragr. J. **2010**, *25*, 150–155

www.interscience.wiley.com/journal/ffj

Table 1. *Continued*

Thymus species	Harvesting place	Four main essential oil components (%)	Oil yield (% v/w)	TBARS	DPPH IC_{50} (mg/ml)	Hydroxyl radical	Superoxide anion inhibition (%)
Thymus capitellatus Hoffmanns. & Link	Alcácer do Sal (MP)	1,8-Cineole (35.0), borneol (16.2), α-pinene (12.4), camphene (11.5)	2.2	1.859 ± 0.123 n	nd	1.231 ± 0.005 jkl	35.9 ± 3.7 l
	Carvalhal (MP)	Borneol (20.3), camphene (18.2), camphor (17.8), α-pinene (12.4)	1.7	0.879 ± 0.032 hij	5.359 ± 0.307 fghij	1.336 ± 0.016 n	40.7 ± 0.8 m
	Santiago do Cacém (MP)	1,8-Cineole (33.7), borneol (16.9), α-pinene (13.6), camphene (11.2)	2.6	0.849 ± 0.049 hij	1.847 ± 0.157 bc	0.989 ± 0.000 hij	33.8 ± 0.2 k
	Estr. Sines-Grândola (MP)	Borneol (22.4), 1,8-cineole (21.1), camphene (16.9), camphor (10.5)	2.0	1.048 ± 0.044 kl	8.112 ± 0.090 i	0.508 ± 0.026 b	51.9 ± 1.4 p
	Tróia (MP)	1,8-Cineole (25.8), borneol (21.0), camphene (12.9), α-pinene (11.1)	3.6	0.801 ± 0.057 gh	nd	0.978 ± 0.001 hij	26.8 ± 1.3 ghi
Thymus carnosus Boiss.	Carvalhal (MP)	Borneol (22.9), camphene (21.1), terpinen-4-ol (11.1), α-pinene (9.7)	0.6	1.174 ± 0.050 lm	4.513 ± 0.083 fg	1.014 ± 0.002 ij	21.2 ± 0.4 cde
	Melides (MP)	Borneol (22.9), terpinen-4-ol (18.3), camphene (16.9), bornyl acetate (6.0)	0.3	0.982 ± 0.025 jk	7.658 ± 0.267 i	1.012 ± 0.001 hij	30.4 ± 0.2 j
	Praia do Barril (MP)	Borneol (26.0), camphene (18.7), terpinen-4-ol (11.1), bornyl acetate (10.2)	1.1	1.145 ± 0.072 ijk	5.903 ± 0.325 h	0.750 ± 0.028 ghij	20.0 ± 0.4 cd
	Quinta do Lago (MP)	Camphene (22.5), borneol (20.1), α-pinene (10.0), bornyl acetate (9.6)	1.1	1.869 ± 0.027 n	10.289 ± 0.380 j	0.965 ± 0.000 d	12.6 ± 0.7 a
	Tróia (MP)	Borneol (21.1), camphene (19.8), terpinen-4-ol (13.6), bornyl acetate (8.0)	0.9	0.757 ± 0.016 gh	5.777 ± 0.029 gh	0.969 ± 0.004 ghij	37.0 ± 0.3 l
	Vila Real de S. António (MP)	Borneol (24.8), camphene (23.7), bornyl acetate (9.5), terpinen-4-ol (8.1)	1.2	0.933 ± 0.054 ijk	4.401 ± 3.285 ef	0.718 ± 0.004 cd	32.9 ± 0.5 k
Thymus pulegioides L.	Carvalhal, Bragança (MP)	γ-Terpinene (25.5), thymol (24.0), carvacrol (22.0), *p*-cymene (7.2)	0.8	0.419 ± 0.006 ef	nd	1.173 ± 0.007 k	27.8 ± 0.5 i
Thymus zygis Loefl. ex L. subsp. *sylvestris*	Alcanena (MP)	Carvacrol (31.8), *p*-cymene (23.5), γ-terpinene (7.7), borneol (6.4)	1.0	0.066 ± 0.003 a	0.453 ± 0.011 a	0.831 ± 0.001 ij	26.9 ± 0.3 ghi
	Condeixa (MP)	*p*-Cymene (35.9), thymol (24.2), γ-terpinene (7.1), borneol (6.4)	0.6	0.078 ± 0.004 ab	0.358 ± 0.005 a	1.015 ± 0.000 e	44.3 ± 0.8 n
	Covão do Coelho (MP)	Carvacrol (34.6), *p*-cymene (24.6), borneol (9.7), camphene (5.3)	1.0	0.073 ± 0.003 ab	0.415 ± 0.024 a	0.902 ± 0.002 fg	50.2 ± 1.0 o
	Duas Igrejas (MP)	*p*-Cymene (39.4), thymol (21.6), γ-terpinene (10.7), linalool (4.3)	0.5	0.087 ± 0.001 ab	0.432 ± 0.008 a	0.948 ± 0.002 fghi	58.8 ± 2.1 q
Thymus zygis Loefl. ex L. subsp. *zygis*	Rebordãos, Bragança (MP)	Carvacrol (43.6), *p*-cymene (24.1), γ-terpinene (15.8), terpinolene (3.2)	0.5	0.030 ± 0.003 a	nd	1.035 ± 0.002 j	39.4 ± 0.5 m

MP, Mainland Portugal; A, Azores; nd, not determined.
Values represent mean ± SD of three independent experiments. Values followed by the same letter in the same column are not significantly different ($p < 0.01$).

control (gallic acid), in which it was possible to determine IC_{50} = 0.035 mg/ml.

In conclusion: (a) no straight correlation can be drawn between dominant essential oil component and antioxidant activity; (b) the oil of a single species can show different antioxidant abilities, depending on its chemotype; and (c) characterization of the antioxidant capacity of an essential oil should be performed using different methodologies of antioxidant assessment, as one oil can show a remarkable antioxidant activity with one methodology and poor with others.

Acknowledgement

This study was partially funded by the Fundação para a Ciência e a Tecnologia (FCT), under research contract PTDC/AGR-AAM/70136/2006.

References

1. J. K. Donnelly, D. S. Robinson. *Free Radic. Res*. **1995**, *22*, 147.
2. N. V. Yanishlieva, E. Marinova, J. Pokorný. *Eur. J. Lipid Sci. Technol*. **2006**, *108*, 776.
3. N. Erdemoglu, N. N. Turan, I. Cakici, B. Sener, A. Aydin. *Phytother. Res*. **2006**, *20*, 9.
4. A. R. Hraš, M. Hadolin, Ž. Knez, D. Bauman. *Food Chem*. **2000**, *71*, 229.
5. C. Sánchez-Moreno. *Food Sci. Technol. Int.* **2002**, *8*, 121.
6. M. Antolovich, P. D. Prenzler, E. Patsalides, S. McDonald, K. Robards. *Analyst* **2002**, *127*, 183.
7. D. Huang, O. Boxin, R. L. Prior. *J. Agric. Food Chem.* **2005,** *53*, 1841.
8. L. K. MacDonald-Wicks, L. G. Wood, M. L. Garg. *J. Sci. Food Agric.* **2006**, *86*, 2046.
9. M. Puertas-Mejía, S. Hillebrand, E. Stashenko, P. Winterhalter. *Flavour Fragr. J*. **2002**, *17*, 380.
10. A. C. Figueiredo, J. G. Barroso, L. G. Pedro, L. Salgueiro, M. G. Miguel, M. L. Faleiro. *Curr. Pharm. Design* **2008**, *14*, 3120.
11. Council of Europe (COE)–European Directorate for the Quality of Medicines. *European Pharmacopoeia*, 6th edn. COE: Strasbourg, **2007**.
12. H. J. D. Dorman, S. G. Deans, R. C. Noble. *J. Essent. Oil Res.* **1995**, *7*, 645.
13. W. Brand-Williams, M. E. Cuvelier, C. Berset. *Lebensm. Wiss. Technol*. **1995**, *28*, 25.
14. S. K. Chung, T. Osawa, S. Kawakishi. *Biosci. Biotechnol. Biochem*. **1997**, *61*, 118.
15. M. Payá, B. Halliwell, J. R. S. Hoult. *Biochem. Pharmacol*. **1992**, *44*, 205.
16. A. Sokmen, M. Gulluce, H. A. Akpulat, D. Daferera, B. Tepe, M. Polissiou, M. Sokmen, F. Sahin. *Food Control* **2004**, *15*, 627.
17. B. Tepe, M. Sokmen, H. A. Akpulat, D. Daferera, M. Polissiou, A. Sokmen. *J. Food Eng*. **2005**, *66*, 447.
18. S. Bounatirou, S. Smiti, M. G. Miguel, L. Faleiro, M. N. Rejeb, M. Neffati, M. M. Costa, A. C. Figueiredo, J. G. Barroso, L. G. Pedro. *Food Chem*. **2007**, *105*, 146.
19. J. Safaei-Ghomi, A. H. Ebrahimabadi, Z. Djafari-Bidgoli, H. Batooli. *Food Chem*. **2009**, *115*, 1524.
20. S. Bourgou, R. Ksouri, I. Skandrani, L. Chekir-Ghedira, B. Marzouk. *Ital. J. Food Sci*. **2008**, *20*, 191.